SYNTHESIS AND STRUCTURE DETERMINATION OF FR109615, A NEW ANTIFUNGAL ANTIBIOTIC

KOHJI KAWABATA, YOSHIKO INAMOTO and KAZUO SAKANE

New Drug Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.,
2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

TOSHIRO IWAMOTO and SEIJI HASHIMOTO

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.,
5-2-3 Tokodai, Tsukuba-shi, Ibaraki 300-26, Japan

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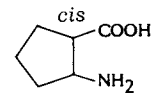
The structure of FR109615, a new antifungal antibiotic, was determined to be (1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid ((−)-*cis*-2-ACPC: **8a**) by X-ray analysis. (−)-*cis*-2-ACPC (**8a**) was also synthesized *via* optical resolution of **3a** and **3b** derived from (±)-*cis*-2-ACPC hydrochloride (**1**). **8a** showed potent antifungal activity, while its antipode (+)-*cis*-2-ACPC (**8b**) had no activity.

FR109615[1], a new antifungal antibiotic, was isolated from *Streptomyces setonii* by our exploratory research laboratories[†,2] (Fig. 1). FR109615 exhibited excellent *in vitro* antifungal activity against *Candida albicans*. FR109615 has mp 195~196°C and $[\alpha]_D^{20}$ −8.9° (*c* 1.0, H_2O), and its structure was elucidated as *cis*-2-aminocyclopentane-1-carboxylic acid (*cis*-2-ACPC), based on the spectroscopic data. Thus FR109615 has a simple and unique structure as compared with the known antifungal substances.

However, the absolute configuration of FR109615 was not reported. In this paper we describe the synthesis and absolute configuration of FR109615.

Fig. 1. Structure of FR109615.



cis-2-ACPC

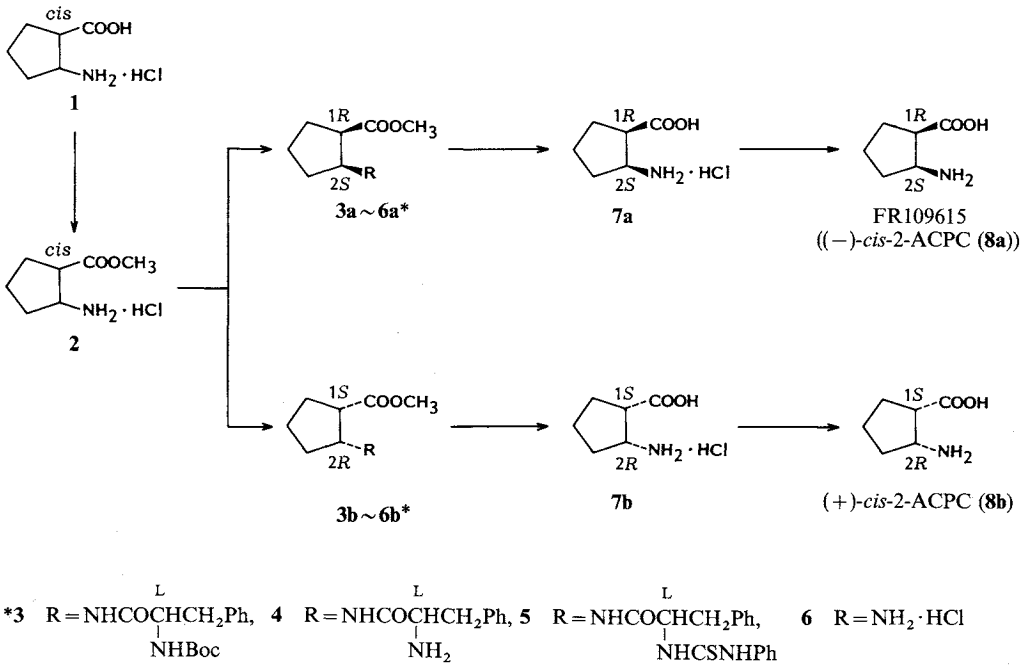
Chemistry

We first synthesized both (−)-*cis*-2-ACPC (**8a**) and (+)-*cis*-2-ACPC (**8b**) by optical resolution of a racemate, (±)-*cis*-2-ACPC hydrochloride (**1**) according to the route as shown in Scheme 1.

The starting material **1** was prepared by the procedure of NATIV and RONA[3]. Esterification of **1** with thionyl chloride in methanol gave the methyl ester **2** in 93.2% yield, which was acylated with *N*-Boc-L-phenylalanine by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSCD) to afford the acylated compound **3** as a mixture of diastereomers. The separation of diastereomers **3** could be performed by fractional crystallizations to give the optical isomers **3a** and **3b** in yields of 18.7 and 30.1%, respectively. Next, EDMAN degradations[4] of each isomer **3a** and **3b** were carried out. Thus, the *N-tert*-butoxycarbonyl (Boc) groups of **3a** and **3b** were removed with hydrogen chloride in ethyl acetate to yield the phenylalanyl derivatives (**4a** and **4b**) in 76.1 and 71.9% yield, respectively. Treatment of **4a** and **4b** with phenyl isothiocyanate gave the phenylthiocarbamoyl derivatives (**5a** and **5b**) in 84.8 and 97.1% yield, respectively. The optical purities of both **5a** and **5b** were confirmed to be >99% enantiomeric excess by HPLC analysis.

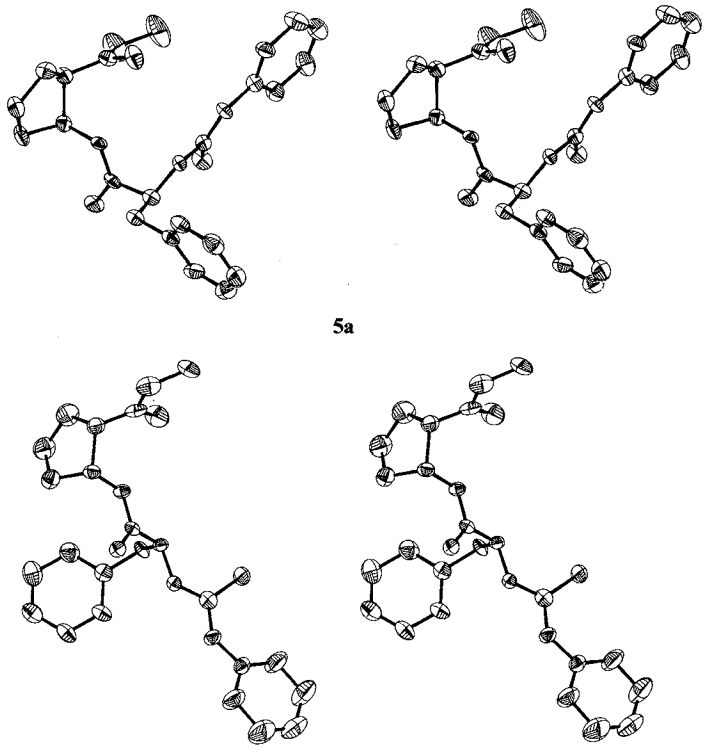
† Bristol-Myers group has recently found an antibiotic identical to FR109615, which they called cispentacin.

Scheme 1.



*3 R = NHCOCHCH$_2$Ph, NHBoc (L) **4** R = NHCOCHCH$_2$Ph, NH$_2$ (L) **5** R = NHCOCHCH$_2$Ph, NHCSNHPh (L) **6** R = NH$_2$·HCl

Fig. 2. Stereoscopic views of **5a** and **5b**.



5a

5b

The absolute configurations of **5a** and **5b** were determined to be (1*R*, 2*S*) and (1*S*, 2*R*), respectively, by X-ray crystallographic analysis (Fig. 2). Thus, the absolute configurations of (**3a**, **4a**) and (**3b**, **4b**) were (1*R*, 2*S*) and (1*S*, 2*R*), respectively.

Optically active (1*R*,2*S*)-2-ACPC (**8a**) and (1*S*,2*R*)-2-ACPC (**8b**) were prepared by deprotection of the *N*-acyl group of **5a** and **5b** with hydrogen chloride, followed by acidic hydrolysis and desalting using an anion exchange resin. The optical purities of both **8a** and **8b** were determined to be >99% enantiomeric excess by HPLC analysis. **8a** has mp 199~200°C and $[\alpha]_D^{20}$ −8.9° (*c* 1.0, H_2O). **8b** has mp 198~199°C and $[\alpha]_D^{20}$ +8.9° (*c* 1.0, H_2O). Since the natural product FR109615 was identical with (1*R*,2*S*)-2-ACPC (**8a**) in all respects, the absolute configuration of FR109615 could be assigned to be (1*R*, 2*S*).

Antifungal Activity

MIC values of FR109615 (**8a**), (+)-*cis*-2-ACPC (**8b**), (±)-*cis*-2-ACPC (**1**) and (±)-*trans*-2-ACPC[5] (**9**) against *Candida albicans* and *Candida tropicalis* are summarized in Table 1.

Only FR109615 (**8a**) showed antifungal activity, and both its antipode **8b** and (±)-*trans*-2-ACPC (**9**) had no activity. It was particularly interesting that the absolute configuration of 2-ACPC is closely correlated with displaying antifungal activity.

Further evaluation on FR109615 will be reported in a separate paper.

Table 1. Antifungal activity of 2-ACPC derivatives (**8a**, **8b**, **1** and **9**) against *Candida albicans* and *Candida tropicalis*.

Compound	MIC (μg/ml)	
	C. albicans FP578	*C. tropicalis* FP583
FR109615 ((−)-*cis*-2-ACPC (**8a**))	6.25	6.25
(+)-*cis*-2-ACPC (**8b**)	>100	>100
(±)-*cis*-2-ACPC (**1**)	12.5	12.5
(±)-*trans*-2-ACPC (**9**)	>100	>100

Experimental

MP's were determined using a Thomas-Hoover capillary melting apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-10 spectrophotometer. Optical rotations were measured with a Jasco DIF-140 automatic polarimeter. NMR spectra were recorded at 90 MHz on a Varian EM-390 NMR spectrometer or a Hitachi R-90H NMR spectrometer using Me_4Si (in $CDCl_3$ and DMSO-d_6) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (in D_2O) as an internal standard.

Antifungal Activity

MICs were determined by the agar dilution method using minimum essential medium (MEM) agar after incubation at 37°C for 18 hours with inoculum size of about 10^6 cfu/ml.

Methyl (±)-*cis*-2-Aminocyclopentane-1-carboxylate Hydrochloride (**2**)

Thionyl chloride (13 ml) was added dropwise to methanol (50 ml) at −15~−5°C, and stirred at the same temperature for 10 minutes. To this solution was added (±)-*cis*-2-ACPC hydrochloride[3] (**1**) (5 g, 30.2 mmol) at −10°C. After being stirred at room temperature for 1.5 hours, the reaction mixture was evaporated *in vacuo* to give 5.05 g (93.2%) of **2** as colorless crystals: MP 120~123°C; IR (Nujol) cm^{-1} 1715, 1595, 1555; ^1H NMR (D_2O) δ 1.68~2.32 (6H, m, $(CH_2)_3$), 3.11~3.33 (1H, m, 1-H), 3.78 (3H, s, CH_3), 3.82~4.02 (1H, m, 2-H).

Anal Calcd for $C_7H_{14}ClNO_2$: C 46.80, H 7.85, Cl 19.73, N 7.80.
Found: C 46.39, H 7.64, Cl 19.32, N 7.76.

Fractional Crystallizations of Methyl (±)-*N*-(Boc-L-phenylalanyl)-*cis*-2-aminocyclopentane-1-carboxylate (**3a**, **3b**)

To a suspension of **2** (15 g, 83.5 mmol) in dichloromethane (200 ml) was added Boc-L-phenylalanine

(22.15 g, 83.5 mmol) and 1-hydroxybenzotriazole (11.28 g, 83.5 mmol) at room temperature. To this mixture was added dropwise 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (14.26 g, 91.85 mmol) under ice-cooling, and the mixture was stirred at the same temperature for 30 minutes. The reaction mixture was poured into water, and the separated organic layer was washed with water, 5% sodium bicarbonate solution and brine, and dried over magnesium sulfate. The organic solvent was evaporated *in vacuo*, and the residue was dissolved in EtOAc (90 ml) at 60~70°C. The solution was allowed to stand at room temperature for 15 hours. The resulting precipitate was collected by filtration to give 9.8 g (30.1%) of **3b** as colorless crystals: MP 132~134°C; $[\alpha]_D^{20}$ +45.6° (*c* 1.0, EtOH); IR (Nujol) cm^{-1} 3330, 3310, 1715, 1680, 1640; ^1H NMR ($CDCl_3$) δ 1.28~2.03 (6H, m, $(CH_2)_3$), 1.41 (9H, s, $(CH_3)_3$), 2.78~3.14 (1H, m, 1-H), 3.01 (2H, d, J=8 Hz, CH_2Ph), 3.58 (3H, s, OCH_3), 4.12~4.55 (2H, m, 2-H +$CHCH_2$), 5.11 (1H, d, J=8 Hz, NH), 6.21 (1H, d, J=8 Hz, NH), 7.27 (5H, s, Ph).

Anal Calcd for $C_{21}H_{30}N_2O_5$: C 64.60, H 7.74, N 7.17.
Found: C 64.63, H 7.56, N 7.22.

The filtrate was evaporated *in vacuo*, and the residue was dissolved in ether (150 ml) under reflux. The solution was left at room temperature for 15 hours, and the resulting precipitate was collected by filtration to give 6.1 g (18.7%) of **3a** as colorless crystals: MP 112~115°C; $[\alpha]_D^{20}$ −63.0° (*c* 1.0, EtOH); IR (Nujol) cm^{-1} 3350, 3330, 1730, 1670, 1650; ^1H NMR ($CDCl_3$) δ 1.41 (9H, s, $(CH_3)_3$), 1.48~2.18 (6H, m, $(CH_2)_3$), 2.78 ~3.08 (1H, m, 1-H), 3.04 (2H, d, J=8 Hz, CH_2Ph), 3.60 (3H, s, OCH_3), 4.18~4.58 (2H, m, 2-H +$CHCH_2$), 4.97 (1H, d, J=8 Hz, NH), 6.64 (1H, d, J=8 Hz, NH), 7.24 (5H, s, Ph).

Anal Calcd for $C_{21}H_{30}N_2O_5$: C 64.60, H 7.74, N 7.17.
Found: C 64.63, H 7.56, N 7.22.

Methyl (1*R*,2*S*)-*N*-(L-Phenylalanyl)-2-aminocyclopentane-1-carboxylate (**4a**)

3a (0.5 g, 1.28 mmol) was added to 4 N hydrogen chloride in EtOAc (1.5 ml) at room temperature, and the mixture was stirred at the same temperature for 15 minutes. The mixture was adjusted to pH 7 with 5% sodium bicarbonate solution, and the resulting precipitate was collected by filtration to give 0.283 g (76.1%) of **4a**: MP 96~101°C; $[\alpha]_D^{23}$ −119.5° (*c* 1.0, $CHCl_3$); IR (Nujol) cm^{-1} 3310, 1730, 1650, 1530; ^1H NMR (DMSO-d_6) δ 1.30~2.03 (6H, m, $(CH_2)_3$), 2.76~3.20 (5H, m, 1-H+NH_2+CH_2Ph), 3.25~3.47 (1H, m, $CHCH_2$), 3.52 (3H, s, CH_3), 4.10~4.52 (1H, m, 2-H), 7.22 (5H, s, Ph), 7.78 (1H, d, J=8 Hz, NH).

Anal Calcd for $C_{16}H_{22}N_2O_3 \cdot \frac{1}{10}H_2O$: C 65.78, H 7.66, N 9.59.
Found: C 65.73, H 7.61, N 9.54.

Methyl (1*S*,2*R*)-*N*-(L-Phenylalanyl)-2-aminocyclopentane-1-carboxylate (**4b**)

3b (60 g, 0.154 mol) was added to 4 N hydrogen chloride in EtOAc (180 ml) at room temperature. The mixture was stirred at the same temperature for 15 minutes, and thereto was added EtOAc (900 ml). The mixture was washed with 5% sodium bicarbonate solution and brine, and dried over magnesium sulfate. The organic solvent was evaporated *in vacuo* to give 32.1 g (71.9%) of **4b** as colorless crystals: MP 72~74°C; $[\alpha]_D^{20}$ +75.0° (*c* 1.0, EtOH); IR (Nujol) cm^{-1} 3400, 3310, 1730, 1640, 1505; ^1H NMR (DMSO-d_6) δ 1.31~2.19 (8H, m, $(CH_2)_3$+NH_2), 2.41~2.62 (1H, m, 1-H), 2.82~3.01 (2H, m, CH_2Ph), 3.28~3.42 (1H, m, $CHCH_2$), 3.54 (3H, s, CH_3), 4.08~4.38 (1H, m, 2-H), 7.21 (5H, s, Ph), 7.70 (1H, d, J=8 Hz, NH).

Anal Calcd for $C_{16}H_{22}N_2O_3$: C 66.19, H 7.64, N 9.65.
Found: C 66.26, H 7.52, N 9.58.

Methyl (1*R*,2*S*)-*N*-(*N*-Phenylthiocarbamoyl-L-phenylalanyl)-2-aminocyclopentane-1-carboxylate (**5a**)

To a solution of **4a** (12.79 g, 44.1 mmol) in EtOH (38 ml) was added phenyl isothiocyanate (7.51 ml, 66.07 mmol) at room temperature. The mixture was refluxed with stirring for 1 hour, and cooled to room temperature. The resulting precipitate was collected by filtration to afford 15.9 g (84.8%) of **5a** as colorless crystals: MP 171~172°C; $[\alpha]_D^{23}$ −66.8° (*c* 1.0, $CHCl_3$); IR (Nujol) cm^{-1} 3320, 1715, 1645, 1630; ^1H NMR (DMSO-d_6) δ 1.33~2.11 (6H, m, $(CH_2)_3$), 2.61~3.28 (3H, 1-H+CH_2Ph), 3.56 (3H, s, CH_3), 4.15~4.52 (1H, m, 2-H), 4.95~5.29 (1H, m, $CHCH_2$), 6.95~7.43 (10H, m, Ph×2), 7.52 (1H, d, J=8 Hz, NH), 8.09 (1H, d, J=8 Hz, NH), 9.66 (1H, br s, NH).

Anal Calcd for $C_{23}H_{27}N_3O_3S$: C 64.92, H 6.39, N 9.87, S 7.53.
Found: C 65.00, H 6.55, N 9.83, S 7.56.

Methyl (1*S*,2*R*)-*N*-(*N*-Phenylthiocarbamoyl-L-phenylalanyl)-2-aminocyclopentane-1-carboxylate (**5b**)

To a suspension of **4a** (30 g, 0.103 mol) in ether (60 ml) was added phenyl isothiocyanate (17.61 ml, 0.155 mol) at room temperature. The mixture was refluxed with stirring for 1 hour, and thereto was added diisopropyl ether (120 ml) at room temperature. The resulting precipitate was collected by filtration to give 42.7 g (97.1%) of **5a** as colorless crystals: MP 125~127°C; $[\alpha]_D^{20}$ +16.5° (*c* 1.0, $CHCl_3$); IR (Nujol) cm^{-1} 3290, 1735, 1710, 1675, 1660; ^1H NMR (DMSO-d_6) δ 1.27~2.07 (6H, m, $(CH_2)_3$), 2.78~3.18 (3H, m, 1-H+CH_2Ph), 3.58 (3H, s, CH_3), 4.08~4.47 (1H, m, 2-H), 4.93~5.25 (1H, m, $CHCH_2$), 6.91~7.59 (10H, m, Ph×2), 7.60 (1H, d, J=8 Hz, NH), 8.02 (1H, d, J=8 Hz, NH), 9.79 (1H, br s, NH).

Anal Calcd for $C_{23}H_{27}N_3O_3$: C 64.92, H 6.39, N 9.87, S 7.53.
Found: C 64.76, H 6.49, N 9.81, S 7.47.

(1*R*,2*S*)-2-Aminocyclopentane-1-carboxylic Acid Hydrochloride (**7a**)

5a (5 g, 11.75 mmol) was added to 4N hydrogen chloride in dichloromethane (25 ml) at room temperature. After being stirred at the same temperature for 15 minutes, the mixture was poured into a mixture of dichloromethane (15 ml) and water (15 ml). The separated aq layer was washed with dichloromethane (45 ml), and thereto was added concd hydrochloric acid (15 ml). The aq solution was warmed to 70°C for 1 hour, and evaporated *in vacuo* to dryness. To the residue was added acetone (30 ml), and the mixture was stirred under ice-cooling for 10 minutes. The resulting precipitate was collected by filtration to give 1.88 g (96.7%) of **7a**: MP 156~157°C; $[\alpha]_D^{20}$ −6.5° (*c* 1.0, H_2O); IR (Nujol) cm^{-1} 3350, 3250, 1710, 1640, 1590, 1500; ^1H NMR (D_2O) δ 1.48~2.43 (6H, m, $(CH_2)_3$), 2.90~3.33 (1H, m, 1-H), 3.67~4.03 (1H, m, 2-H).

Anal Calcd for $C_6H_{11}NO_2 \cdot \frac{3}{5}H_2O$: C 40.85, H 7.55, Cl 20.09, N 7.93.
Found: C 40.98, H 7.63, Cl 19.58, N 7.97.

(1*S*,2*R*)-2-Aminocyclopentane-1-carboxylic Acid Hydrochloride (**7b**)

7b was obtained (83.3%) from **5b** in a similar manner to that as described for the synthesis of **7a**: MP 156~157°C, $[\alpha]_D^{20}$ −6.5° (*c* 1.0, H_2O); IR (Nujol) cm^{-1} 3360, 3230, 1715, 1650; ^1H NMR (D_2O) δ 1.52~2.33 (6H, m, $(CH_2)_3$), 2.81~3.33 (1H, m, 1-H), 3.72~4.01 (1H, m, 2-H).

Anal Calcd for $C_6H_{11}NO_2 \cdot \frac{3}{5}H_2O$: C 40.85, H 7.55, Cl 20.09, N 7.93.
Found: C 40.86, H 7.53, Cl 19.92, N 8.00.

(1*R*,2*S*)-2-Aminocyclopentane-1-carboxylic Acid (**8a**)

A solution of **7a** (25 g, 0.151 mol) in water (125 ml) was adjusted to pH 6.7 with an anion exchange resin Diaion SA10A (OH^-) at room temperature. The resin was filtered off, and the filtrate was evaporated *in vacuo* to dryness. To the residue was added EtOH (125 ml) and diisopropyl ether (250 ml) with stirring under ice-cooling. The resulting precipitate was collected by filtration to give 16.12 g (82.7%) of **8a** as colorless crystals: MP 199~200°C (dec); $[\alpha]_D^{20}$ −8.9° (*c* 1.0, H_2O); IR (Nujol) cm^{-1} 2950, 2200, 1640, 1550; ^1H NMR (D_2O) δ 1.52~2.30 (6H, m, $(CH_2)_3$), 2.67~3.05 (1H, m, 1-H), 3.47~3.87 (1H, m, 2-H).

Anal Calcd for $C_6H_{11}NO_2$: C 55.80, H 8.58, N 10.84.
Found: C 55.52, H 8.44, N 10.65.

FR109615 was identical with **8a** in all respects.

(1*S*,2*R*)-2-Aminocyclopentane-1-carboxylic Acid (**8b**)

8b was obtained (83.3%) from **7b** in a similar manner to that as used for the synthesis of **8a**: MP 198~199°C (dec); $[\alpha]_D^{20}$ +8.9° (*c* 1.0, H_2O); IR (Nujol) cm^{-1} 1640, 1540, 1460; ^1H NMR (D_2O) δ 1.57~2.18 (6H, m, $(CH_2)_3$), 2.71~2.99 (1H, m, 1-H), 3.57~3.82 (1H, m, 2-H).

Anal Calcd for $C_6H_{11}NO_2 \cdot \frac{1}{20}H_2O$: C 55.41, H 8.60, N 10.77.
Found: C 55.43, H 8.59, N 10.67.

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